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Effects of different blanching treatments on colour and microbiological profile of *Tenebrio molitor* and *Zophobas morio* larvae

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ABSTRACT

This study aims to evaluate the effects of two blanching treatments (60 °C for 5 min and 90 °C for 1 min) and of different solutions' pH (unmodified and standardized pH at pH 2, 4, 6 and 8) on qualitative parameters such as enzymatic browning and microbiological parameters of two edible insect species, *Tenebrio molitor* and *Zophobas morio*. Moreover, fresh larvae (unblanched) were compared with larvae that were subjected to two heat treatments. Results showed that the use of blanching treatments and different pH of the solution can slow down and even block enzymatic browning. In both species the enzymatic browning decreased after the blanching treatments and also the acidification at pH 2 showed a positive action, due to the inactivation of the enzymes responsible for the browning effect. In addition, the blanching treatments influenced the microbiological parameters; in fact, they were able to reduce the total bacterial load in both species, compared to fresh larvae, increasing the safety and hygienic quality of the larvae. The results of the present research could be useful for the setting up of new products containing edible insects.

1. Introduction

Edible insects represent a viable sustainable alternative source to answer the increasing demand of nutritional foods and proteins in relation to the growing human population (van Huis et al., 2013). The rearing of insect showed many advantages when circular economy concept is applied, in fact they can be farmed on different substrates (feed), as by-products/waste of the agri-food industries, which would not have another use. In addition, insects as cold-blooded animals are able to transform almost all the feed they eat into body mass. Edible insects is a wide category represented by a great variety of species, however in general, insects are rich in proteins, followed by fats, vitamins, minerals and fibers (Kouřimská & Adámková, 2016). It has been observed that *Tenebrio molitor* farming require low quantity of water and less space than conventional farmed animals (Oonincx & de Boer, 2012), furthermore emit less greenhouse gas amount (Oonincx et al., 2010; van Broekhoven, Oonincx, van Huis, & van Loon, 2015; van Huis et al., 2013). *Tenebrio molitor* (mealworm) is one of the most studied insects for food and feed purposes thanks to its balanced chemical composition, as it showed to be rich in both proteins (50% on dry matter, DM) and lipid (30-34% on DM) (Mancini, Fratini, Turchi, et al., 2019; Osimani et al., 2017). Furthermore, it is also an excellent source of essential amino acids, vitamins and minerals (Finke, 2015). Another beetle, belonging to the Tenebrionidae family, is *Zophobas morio*, also known as giant mealworm or superworm. Similarly, to mealworm also superworm larvae show an excellent chemical composition, with 46% protein (on DM) and 43% lipid (on DM) (Soares Araújo et al., 2019). Nevertheless, there are several obstacles that limit the use of insects as food, especially in countries were entomophagy is no more a common practice. For example, European consumers may show some reticence while consuming insects (Mancini, Moruzzo, Riccioli, & Paci, 2019). Tan, van den Berg, and Stieger (2016) investigated the acceptance or rejection factors of insect-based food. Their results showed that (i) the visibility of the insect decreases the intention of consumption, (ii) savory products were preferred, probably because insects are seen as an alternative to meat and, finally (iii) they showed how one of the most important factors is the familiarity of the product, primarily in appearance and later in taste. For these reasons, it is essential that food products containing

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insects as an ingredient fulfil the sensory, quality and safety expectations of the consumers. Insects are added to well-known products as powder, therefore technological processing must be undertaken to breakdown the insect body. Research studies reported that mechanical breakage exposes several compounds contained in the insect to oxidation, causing the formation of molecules that might affect the sample characteristics, from the view of both nutritional and quality aspects (Janssen, Vincken, Arts, Fogliano, & Lakemond, 2019, 2017; Mancini, Fratini, Tuccinardi, et al., 2019; Ssepuuya et al., 2020; Tonneijck-Srpová, Venturini, Humblet-Hua, & Bruins, 2019). These modifications could affect sensory characteristics of the product, such as the colour which is crucial to maintain consumers acceptance and increase visual sensory evaluation of insect-based products. Browning is one of the main issues in several edible insect species, indeed mealworm, lesser mealworm, black soldier fly and long-horned grasshopper could face this colour modification during processing (Janssen et al., 2017; Mancini, Fratini, Tuccinardi, et al., 2019; Ssepuuya et al., 2020; Tonneijck-Srpová et al., 2019). Interestingly Roncolini et al. (2019), observed that bread formulated with 5% or 10% of mealworm powder modified its colour in relation to the insect percentage, making these types of insect-based bread visually different from the original product. The browning of a food product could often be linked to its deterioration, therefore a decrease of its biological value and safety (Friedman, 1996). Several enzymes play a role in browning, such as phenoloxidase, laccase, tyrosine hydroxylase, decarboxylase and peroxidase (Andersen, 2012). In insects most of the phenolic compounds are derive from L-tyrosine produced in the shikimic acid pathway and are often subject to modifications by enzymes. Furthermore, phenolic compounds can react, through non-enzymatic reactions, with proteins and amino acids, forming dark compounds (melanins). Janssen et al. (2017) reported the enzymatic activity on phenols of three insect species (*Tenebrio molitor*, *Alphitobius diaperinus*, *Hermetia illucens*) highlighting that the highest enzymatic activities were observed in the pH range 6–7 which corresponds with the physiological pH of the three species. No enzymatic browning was observed in all three species at pH 4. Blanching is a treatment used in the food industry with the aim of deactivating enzymes. In this process, samples are blanched for a short time in hot water or steam at temperatures ranged between 70 and 150 °C. Mancini, Fratini, Tuccinardi, et al. (2019), observed that the browning in mealworm larvae, were completely blocked by blanching the larvae at 60 °C for 5 min, confirming the hypothesis that this variation is mainly linked to the total or partial loss of the enzymatic activity of the phenoloxidase, altered by the heat treatment. In addition, a correlation between pH of the minced and blanched samples and change in colour (6 h) was reported. On the other hand, the blanching heat treatment can also increase safety of the products, affecting the microbial loads (Fellows, 2009; Wang et al., 2017). Indeed, Mancini, Fratini, Tuccinardi, et al. (2019) showed that blanching at 60 °C for 5 min or at 90 °C for 2.5 min reduced the microbial loads of mealworm larvae. Similarly, Wynants et al. (2018) and Vandeweyer, Lenaerts, Callens, and Van Campenhout (2017), respectively, noted that blanching at 88 °C for 5 min significantly reduced the bacterial loads in *Alphitobius diaperinus*, as well as a significant reduction was showed in all microbial counts of blanched *Tenebrio molitor* larvae at 100 °C for 10, 20 and 40 s. The aims of the present research were to investigate the effects of (i) blanching treatments (60 °C for 5 min and 90 °C for 1 min) and (ii) the solutions pH (unmodified and standardized at pH 2, 4, 6 and 8) on the enzymatic browning and microbiological parameters of *Tenebrio molitor* and *Zophobas morio* larvae.

2. Materials and methods

2.1. Rearing conditions and blanching treatments

Tenebrio molitor (mealworm) and *Zophobas morio* (superworm) were reared at the Department of Veterinary Sciences of the University of Pisa (Italy) under a close circle laboratory scale production. Mealworms and superworms were reared at 25 °C, relative humidity 50-60%, in plastic containers (39 × 28 × 14 cm) and fed a mix (1:1) of bread and brewery spent grains. Bread was collected from a market shop as daily remains (leftovers) while brewer's spent grains were directly collected from a local brewery and frozen at −20 °C. Bread and spent grains were dried in an oven at 90 °C to remove excessive humidity (spent grains were previously thawed for 18 h at 4 °C). Both bread and brewery spent grains were finely ground before mixing them. Twice a week potato slices were added to provide moisture. The mealworms were harvested when the first pupa was observed (larva weight about 150 mg), while superworms were collected at the final larvae stage when no more growth was showed (larvae weight about 1 g). Larvae were fasted for 48 h in sterile plastic containers with plastic web on the base in order to separate faeces and to avoid faecal contact, then they were killed by freezing at −18 °C (for the microbiological analyses the larvae were frozen for only 1 h).

Larvae were tested as unblanched (control, C), blanched in water at 60 °C for 5 min (B60_5) and blanched in water at 90 °C for 1 min (B90_1). Blanching was performed in glass recipients with a water:insect ratio of 50:1 (v:w). After blanching larvae were cooled in cold (4 °C) water to interrupt heat processes.

All trials were repeated three times on three different insect batches derived from different boxes.

2.2. Colour determination

In order to test enzymatic browning process (Janssen et al., 2017; Mancini, Fratini, Tuccinardi, et al., 2019; Yi et al., 2013) C, B60_5 and B90_1 were homogenized in five different solvents prior to the colour analyses (solvent:insect ratio 10:1, v:w). Larvae were homogenized with and Ultra Turrax (Polytro PT 3000, Kinematica AG, Eshbach, Germany) in distilled water (dw), HCl solutions at pH 2, 4 and 6 (pH2, pH4 and pH6) and NaOH solution at pH 8 (pH8). Right after homogenising debrides and macroscopic parts were removed via plastic sieve. Colour were determined on all the fifteen samples (3 blanching treatments × 5 solutions) per each insect species up to 360 min with an interval time of 10 min up to 90 (0, 10, 20, 30, 40, 50, 60, 70, 80 and 90) and then an interval time of 30 min (120, 150, 180, 210, 240, 270, 300, 330 and 360).

Colour was measured by pouring the homogenates in plastic vials via Minolta CR300 chroma meter (Minolta, Osaka, Japan) with an aperture size of 8 mm, illuminant D65 and incidence angle of 0°, recording the L* (lightness), a* (redness) and b* (yellowness) indexes, according the CIElab system (CIE, 1976).

Basing on L*, a* and b* indexes the whiteness (WI), yellowness (YI) and browning (BI) indexes were calculated according to Rhim, Wu, Weller, and Schnepf (1999) and van Huis and Tomberlin (2016) following the formula:

$$WI = \sqrt{\left(100 - L^{*2}\right) + a^{*2} + b^{*2}}$$

$$YI = \frac{142.86 \times b^*}{L^*}$$

$$BI = 100 \times \frac{X - 0.31}{0.17} \; where \;\; X = \frac{a^* + 1.75 \times L^*}{5.645 \times L^* + a^* - 3.012 \times b^*}$$

Colour differences were calculated between time 0 and the following colour analysis times for each combination of the insect-blanching-solution following the formula reported by Sharma and Bala (2002):

$\Delta E = \sqrt{(L_0^* - L_\beta^*)^2 + (a_0^* - a_\beta^*)^2 + (b_0^* - b_\beta^*)^2}$ where β represent time 10, 20, 30, 40, 50, 60, 70, 80, 90, 120, 150, 180, 210, 240, 270, 300, 330 and 360.

2.3. Microbiological profile

For microbiological analyses 10 g of larvae (C, B60_5 and B90_1, both *Tenebrio molitor* and *Zophobas morio*) were diluted in 90 ml of sterile saline solution in sterile plastic bags. Larvae were then hand cracked and the mixture was subsequently homogenized via a stomacher for 60 s (Stomacher® 400 Circulator, VWR International Sr). Ten-fold dilution were then plated on plate count agar (PCA, ThermoFisher Scientific, Milan, Italy) for the quantification of the total viable aerobic count (incubated at 30 °C for 72 h). Microbial counts were expressed in log colony-forming unit (CFU)/g as mean of three replicates.

2.4. Statistical analysis

Experimental design of the research trail is reported in Fig. 1. Colour data of each insect species were statistically analysed following the linear model $Y_{ijkz} = \mu + \alpha_i + \beta_j + \gamma_k + \alpha\beta_{ij} + \alpha\gamma_{ik} + \beta\gamma_{jk} + \alpha\beta\gamma_{ijk} + e_{ijkz}$, where Y_{ijkz} is the dependent variable of the zth observation; μ is the overall mean; α_i is the effect of the blanching (i = C, B60_5 and B90_1); β_j is the effect of the solution (dw, pH2, pH4, pH6 and pH8); γ_k is the effect of the time (0, 10, 20, 30, 40, 50, 60, 70, 80, 90, 120, 150, 180, 210, 240, 270, 300, 330 and 360); $\alpha\beta_{ij}$, $\alpha\gamma_{ik}$, $\beta\gamma_{jk}$ and $\alpha\beta\gamma_{ijk}$ are the effects of the interaction between the main factors and e_{ijkz} is the random error. Effect of blanching (C, B60_5 and B90_1) on the microbiological profile of the larvae was tested for both the insect species via a one-way ANOVA.

The significance level was set at 5%, differences between means were compared using Tukey's procedure and the variability was expressed as Root Mean Square Error (RMSE). R software was used (R Core Team, 2015).

3. Results and discussion

Food appearance is a crucial characteristic that affects the acceptability of the consumer.

Colour parameters p-values of the linear model were reported in Table 1. All the colour parameters showed to be affected by the single main factors and interactions Blanching × Solution and Blanching × Time. No significative p-values were reported for interaction s Solution × Time and Blanching × Solution × Time. Both the insect species showed the same p-value patterns of significancy. For consistency the lightness (L*), redness (a*) and yellowness (b*) values of unblanched (C) and blanched (B60_5 and B90_1) mealworms and superworms larvae submitted to the different pH solutions and analysed at the different times are reported in Fig. 2 (mealworms) and Fig. 3 (superworms) recording the effect of blanching.

Lightness of unblanched larvae (Fig. 2a) decreased over time with major changes in the first 90 min, then slightly slowed the reducing trend. The solutions' pH affected the L* parameter as mainly revealed by pH2 and partially by pH4 in the first 180 min. At the end of the trial while pH2 reached L* value of 54.14 the other solutions were ranged between 36.63 and 37.38. Also other Authors observed that the L* values of fresh larvae were the lowest compared to larvae subjected to blanching (Mancini, Fratini, Tuccinardi, et al., 2019; Tonneijck-Srpová et al., 2019). As reported by Janssen et al. (2017), when *Tenebrio molitor* larvae are grinded they undergone enzymatic and not-enzymatic modifications that lead to a browning of the paste. Indeed, after grinding the phenol oxidase could hydrolase monophenols with the production of *ortho*-diphenols, which then oxides to *ortho*-quinones. Then quinones react, via a non-enzymatic pathway, with proteins formatting melanins which contribute to the final browning of the larvae paste. Samples at pH 2 showed the highest L* values, in agreement with Mizobutsi et al. (2010) who observed a complete inactivity of peroxidases and polyphenol oxidases at pH values equal to 2.5. Indeed, in our study the unblanched samples showed to maintain quite the same L* value when treated at pH 2. Furthermore, it is in agreement with what was stated by Mancini, Fratini, Tuccinardi, et al. (2019) who documents how the change in colour is linked to changes in pH, as it affects the enzymatic activity and also the characteristics of the organic compounds present.

The two heat treatments reduced drastically the differences between the different solutions, with a mild solution-time effect in B60_5 samples (Fig. 2b) and a very low effect in the B90_1 samples (Fig. 2c). Redness parameter revealed to be less influenced by the solutions employed,

Table 1
Effects of blanching, solution, time, and their interactions (p-values and Root Mean Square Errors) on the colour parameters of mealworm and superworm larvae.

Factors	Mealworm			Superworm		
	L*	a*	b*	L*	a*	b*
Blanching	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Solution	<0.001	0.012	<0.001	<0.001	<0.001	<0.001
Time	<0.001	<0.001	<0.001	<0.001	0.181	<0.001
Blanching × Solution	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Blanching × Time	<0.001	<0.001	<0.001	<0.001	0.001	0.002
Solution × Time	0.643	1.000	0.987	1.000	0.937	1.000
Blanching × Solution × Time	0.882	1.000	0.758	1.000	1.000	1.000
RMSE	2.336	0.377	0.786	2.703	0.325	0.842

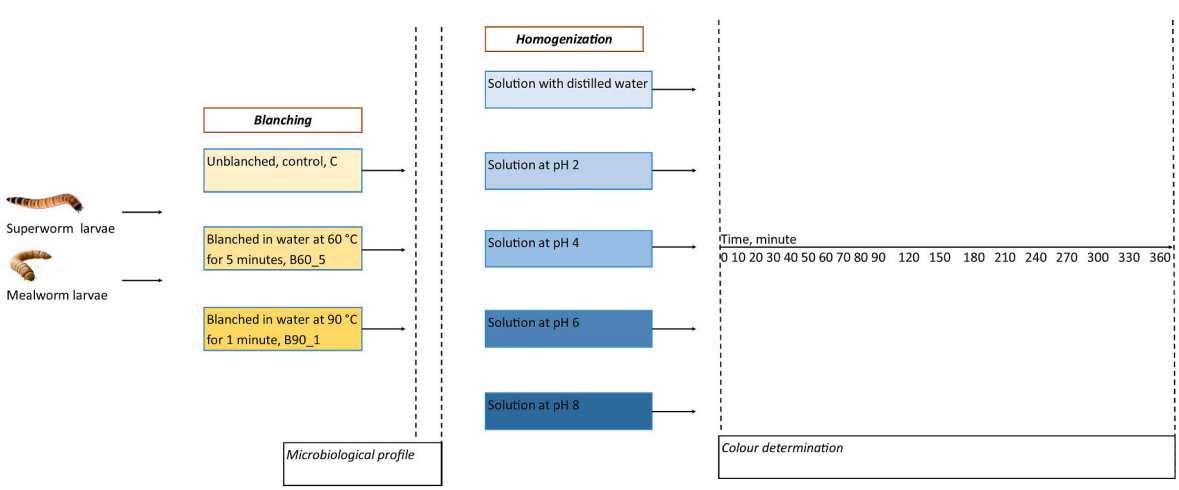

Fig. 1. Experimental design.

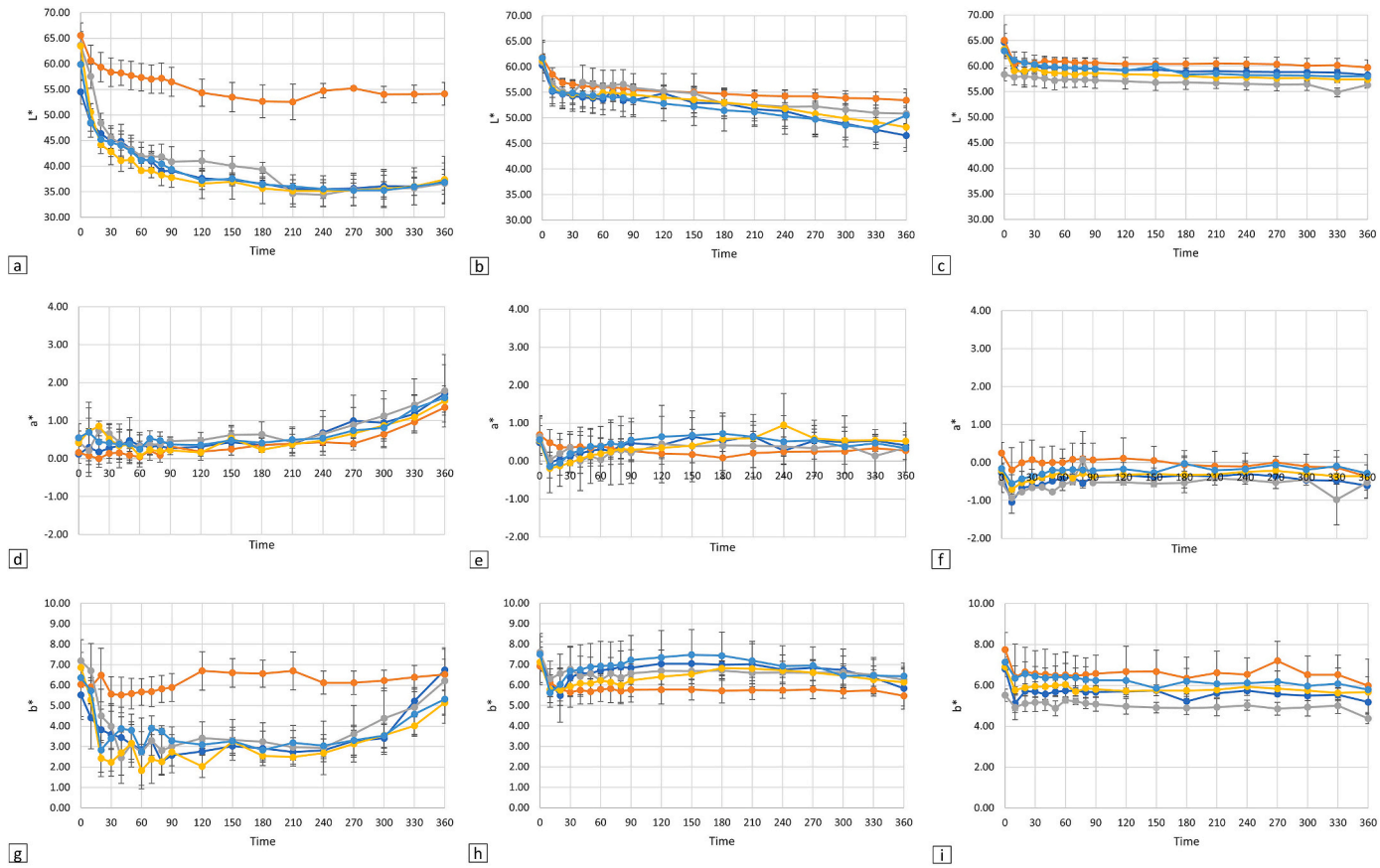

Fig. 2. Colour indices of unblanched (C) and blanched (B60_5 and B90_1) mealworms larvae.
Lightness (L*), redness (a*) and yellowness (b*) values (mean ± sd) of unblanched (graphics a, d, and g), blanched at 60 °C for 5 minutes (graphics b, e and h) and blanched at 90 °C for 1 minute (graphics c, f and i) mealworms larvae. Lines represent solution in distilled water (dw) or solution at pH 2, 4, 6 or 8. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

meanwhile, was affected by the blanching steps. Indeed, redness of larvae blanched at B60_5 and B90_1 did not show a wide modification during time (Fig. 2e and 2f, respectively), while unblanched larvae increased their a* value after 210-240 min (Fig. 2d). Yellowness parameter, as reported by L*, was affected by pH in unblanched larvae (Fig. 2g). Unblanched larvae homogenized in solution at pH 2 showed to maintain a quite stable b* value while the other samples reported to decrease the value in the first half part of the trial followed by an increase of b* in the second part. Indeed, even if the samples showed different patterns during time the final values of all the solutions were comparable. Heat treatments reduced the variability between the solutions although a highest variance between the samples was showed in B90_1 then in the other colour parameters of the same treatment (Fig. 2i). The enzymes are also inactivated by high temperatures, for this reason in the samples B60_5 and B90_1 the redness parameter did not show evident variations compared to the unblanched larvae. These data are in agreement with Saucier et al. (2021) who reported that a pre-treated in boiling water significantly reduce the darkening effect on the subsequent freeze-drying. Moreover, Seho et al. (2021) reported that blanching followed by vacuum or multiflash drying methods decreases browning and colour changes in mealworm larvae.

Furthermore, these data are in line to the ones reported by Mancini, Fratini, Tuccinardi, et al. (2019) which observed very few variations in the a* parameter of the samples treated at 80 °C and 90 °C.

Lightness of unblanched superworm larvae showed to be affected both by pH solution and time (Fig. 3a). All the samples decreased during time their L* value with a main difference between time 0 and 10 min, then a constant low trend was showed. Solution at pH 2 maintained higher values of L* during all the trial with final value of 58.40 while the other solutions were ranged between 48.06 and 51.86. As observed by de Oliveira Carvalho and Orlanda (2017) enzyme activity of polyphenol oxidase in buriti (*Mauritia flexuosa* L.) fruit extract could be completely deactivated at pH 2. Blanched larvae for 5 min at 60 °C reported a similar trend in relation to the time-solutions with decreasing L* values. Notably, solutions did not gather all together in terms of L* values but a gradient of pH effect was showed. Solution pH2 samples reported the higher L* values followed by pH4, pH6 and dw solutions then by pH8 (Fig. 3b). Differently, Vandeweyer et al. (2017) did not shown enzymatic activity at pH 4 for *Tenebrio molitor*, *Alphitobius diaperinus*, *Hermetia illucens*. These effects were not evident in larvae blanched at 90 °C for 1 min as, after an initial decrement of L* for all the samples, the L* values remained quite stable during all the trial (Fig. 3c). According to Adams (1991), the structure of the enzyme changes on the basis of the matrix and of the micro-environmental factors than consequently also its sensitivity to inactivation could be affected as well, however in animal tissues the deactivation occurs at the temperature range of 50–70 °C.

Redness of unblanched superworm larvae showed different trends in relation to the solution adopted for the samples during the time (Fig. 3d). While dw, pH4, pH6 and pH8 samples increased their a* values during the time, pH2 remained stable from minute 10 to the end of the trial after the initial decrease. All the solutions showed this trend to maintain the same a* value during all the trial in B60_5 and B90_1 samples, again with the lowest values showed by solution pH2 samples (Fig. 3e and 3f). Effects of solutions in yellowness parameter (b*) of unblanched and blanched larvae (Fig. 3g, 3h and 3i, respectively for C, B60_5 and B90_1) reported significant differences between the single solutions but a common trend. Unblanched larvae decreased the b* values during the first part of the trial then the index increased by the

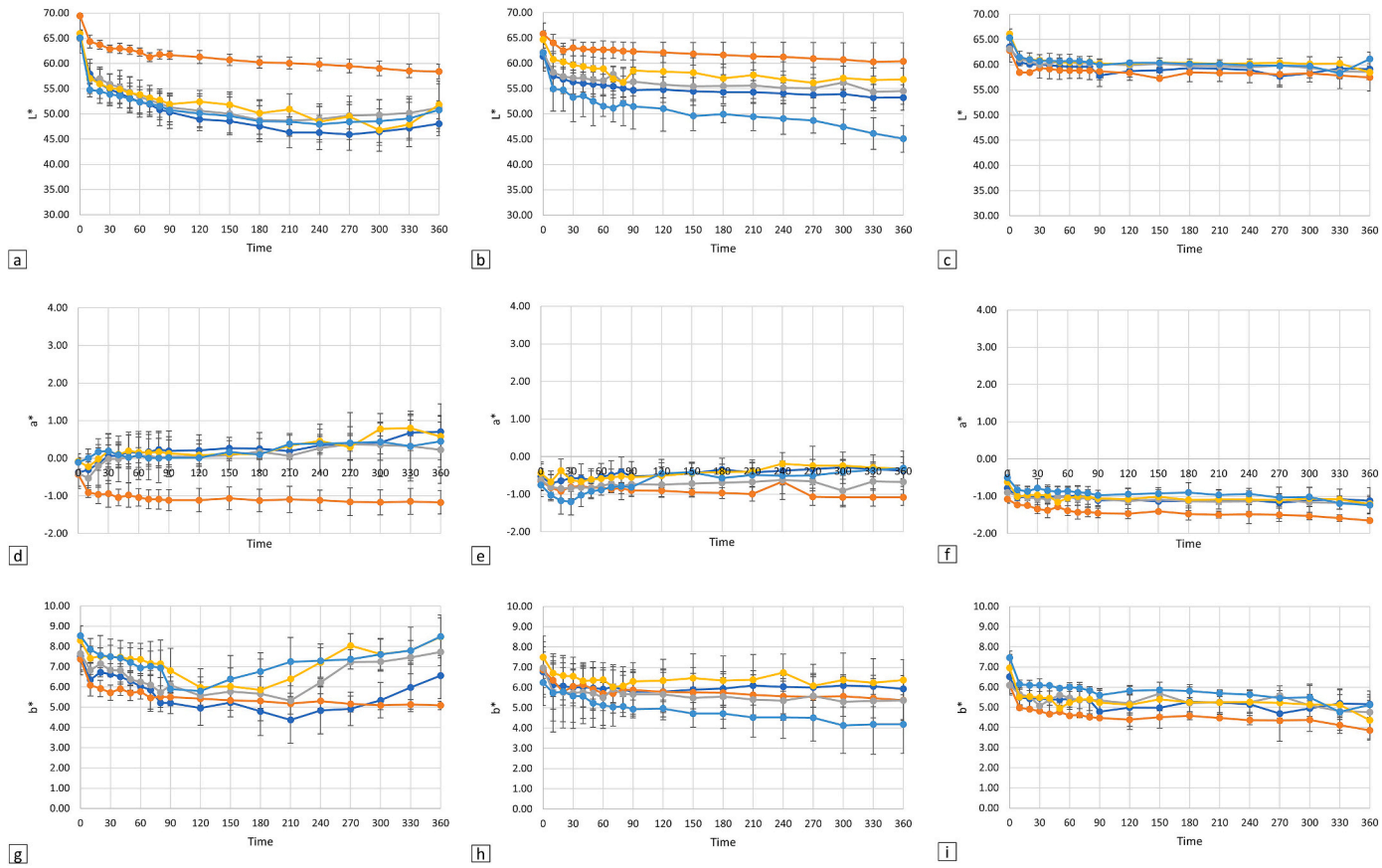

Fig. 3. Colour indices of unblanched (C) and blanched (B60_5 and B90_1) superworms larvae.
Lightness (L*), redness (a*) and yellowness (b*) values (mean ± sd) of unblanched (graphics a, d, and g), blanched at 60 °C for 5 minutes (graphics b, e and h) and blanched at 90 °C for 1 minute (graphics c, f and i) superworms larvae. Lines represent solution in distilled water (dw) or solution at pH 2, 4, 6 or 8. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

end. This effect was not highlighted in blanched larvae where the b* parameter maintained values between 4.13 and 7.49 or between 3.86 and 7.47 respectively for B60_5 and B90_1 samples.

Whiteness index (WI), colour differences (ΔE) and browning index (BI) of both mealworm and superworm larvae submitted to the different treatments are reported in Fig. 4.

WI of mealworm (Fig. 4a) showed a variability in relation to the blanching step. Larvae blanched for 1 min at 90 °C reported the higher WI value, followed by B60_5 and C. Only WI values of pH 2 unblanched samples showed difference from the other C samples, with values more ascribable to the B60_5. A similar trend was showed by superworms, with minor differences between samples C and B60_5. Colour differences (ΔE), calculated as difference in colour between time 0 and the following times, highlighted as the main effect the blanching treatment followed by the effect of pH. Also, Saucier et al. (2021) reported a strong effect of blanching on black soldier fly larvae. Indeed, Authors reported that freeze-dried samples that were blanched for 40 s showed the least colour change compared to the raw-thawed larvae, moreover this colour changes were lower than samples scalded in boiling water for 8 min and those that were punctured (50 μm holes). The same results were also reported in colour variation prior to the drying step, in which blanched larvae showed a lower variation than the other treatments (scalding for 2, 4, 6 and 8 min). Superworm samples showed a lower variation in ΔEs, with overlapping between C and B60_5 samples (mostly at pH 2 and 4, Fig. 4d). BI showed the efficacy of treatment at 90 °C for 1 min for both the insects, while blanching at 60 °C showed only in superworm an effect in retarding browning (Fig. 4e and 4f, respectively for *Tenebrio molitor* and *Zophobas morio* larvae). Yellowness indexes (YI) were not reported in graphics as the values followed principally the trends reported for b* values as the influence of L* in the formula was quite low due to the common trends of the samples.

Microbiological quantifications of the total viable aerobic count of mealworm and superworms larvae unblanched and blanched are reported in Fig. 5.

Unblanched mealworm larvae showed a total viable aerobic count of 7.22 log CFU/g, which statistically decreased after blanching for 5 min at 60 °C at 5.74 log CFU/g. Blanching the larvae at 90 °C for 1 min induced a total reduction of the total viable aerobic count to a value under the limit of detection of the method. Superworm larvae showed a reduction of the total viable aerobic count from 6.84 log CFU/g of unblanched samples to 4.64 log CFU/g of the B60_5 ones. No further effect was showed by the blanching at 90 °C for 1 min as the microbial count lied in the same range of contamination (4.57 log CFU/g). This lack of further action by the higher blanching temperature could be ascribed to the size of the insect larvae that did not lead the reach an adequate core temperature. Anyway, the two heat treatments were effective in reducing total bacterial count in both *Tenebrio molitor* and *Zophobas morio*. According to Wang et al. (2017) and Fellows (2009) blanching is a technological process capable of reducing the microbial load.

The microbiological data obtained in this study are also in agreement with Mancini, Fratini, Tuccinardi, et al. (2019) who observed a significant reduction in the total bacterial count in *Tenebrio molitor* larvae blanched at 60 °C for 5 min and also after blanching at 90 °C for 2.5 min. Wynants et al. (2018) observed that blanching at 88 °C for 5 min significantly reduced bacterial loads in *Alphitobius diaperinus*. Furthermore, Vandeweyer et al. (2017) observed a reduction in all the microbial counts evaluated in blanched *Tenebrio molitor* at 100 °C for 10, 20 and

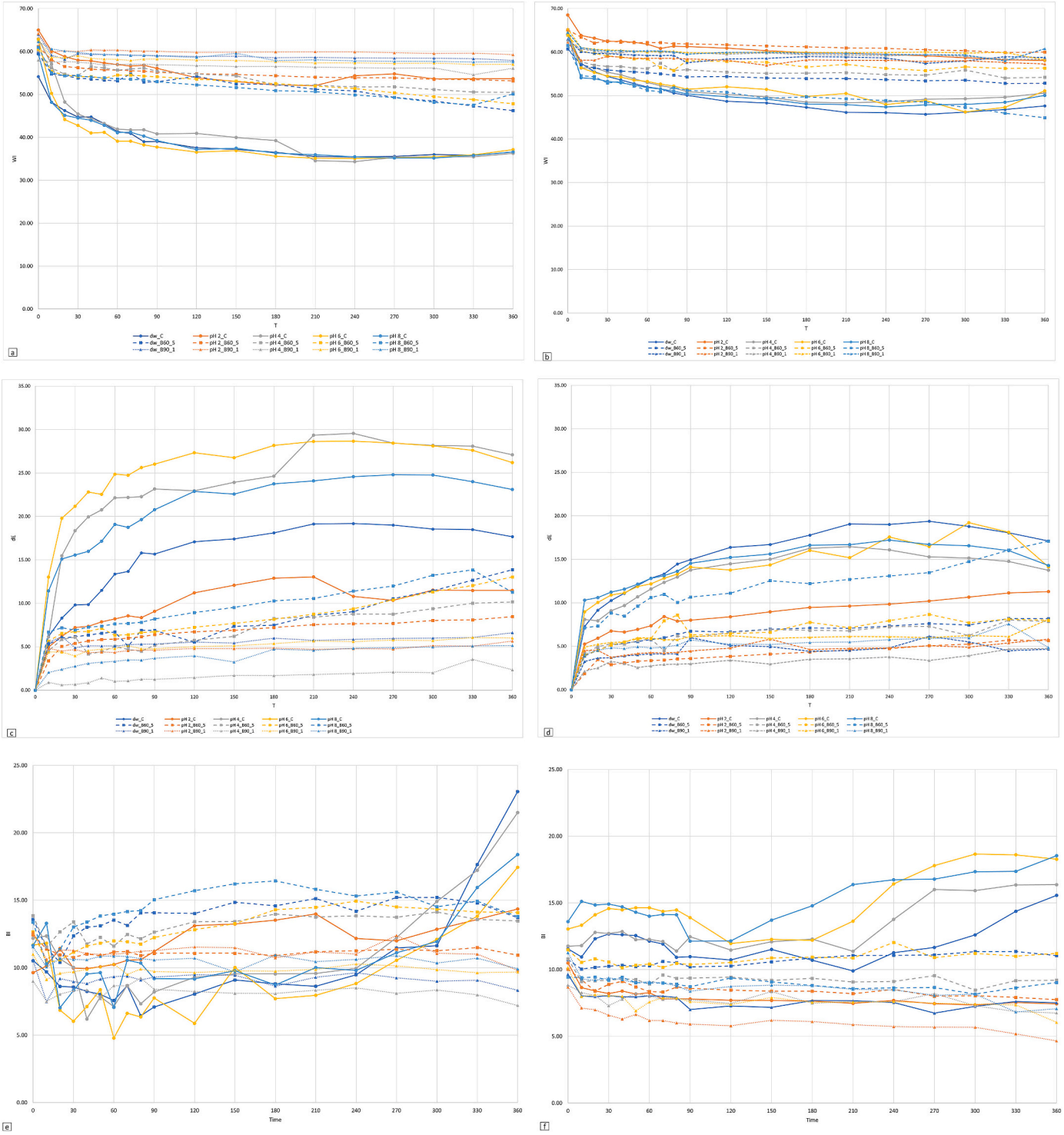

Fig. 4. Whiteness index (WI), colour differences (ΔE) and browning index (BI).
Graphics a, c, and e: mealworm larvae. Graphics b, d, and f: superworm larvae. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

40 s. Also, Saucier et al. (2021) reported a reduction in the microbial load in black soldier fly larvae pre-treated with blanching.

4. Conclusions

Colour is one of the main drivers in consumer acceptance, mostly in relation to the willingness to purchase a new product. In both the tested insect species, *Tenebrio molitor* and *Zophobas morio*, the blanching treatments at 60 °C for 5 min and 90 °C for 1 min allowed to block the enzymatic browning process. An effect of the pH was also highlighted, in particular pH 2 in blocking enzymatic browning. Furthermore, the heat treatments were effective in lowering total bacterial count, an important factor for the hygienic quality of the product. These results are a starting point for obtaining new food products, containing insect, at least respect the visually qualities expected by the consumers.

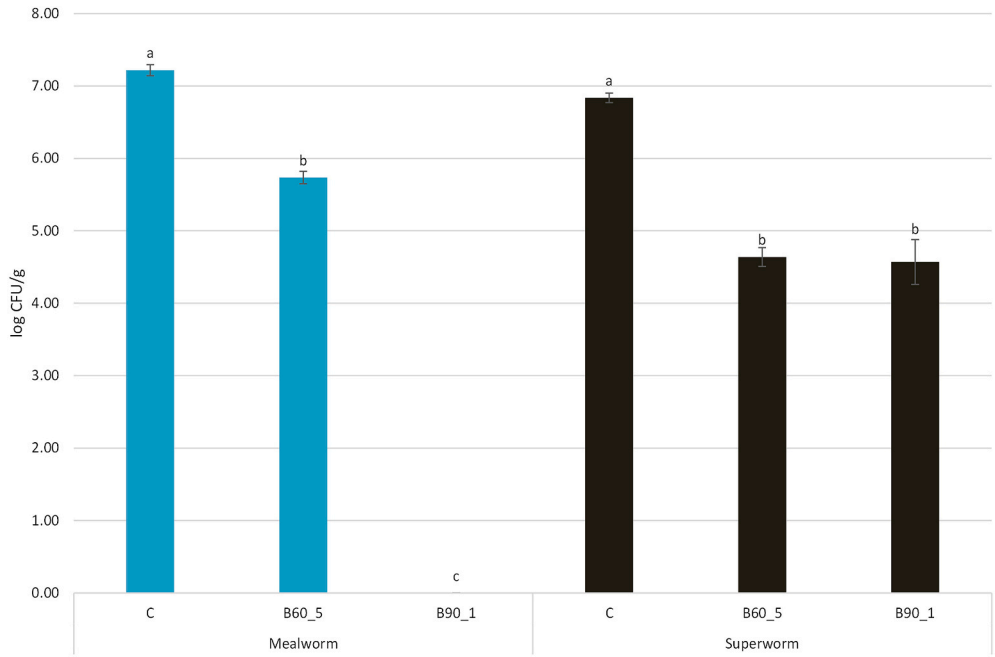

Fig. 5. Total viable aerobic count (log CFU/g) of mealworm and superworms larvae unblanched (C) and blanched at 60 °C for 5 min (B60_5) or at 90 °C for 1 min (B90_1).
Superscript a, b and c indicate significant differences at p-value < 0.05 for each treatment (C, B60_5 and B90_1) per insect species.

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CRediT authorship contribution statement

Chiara Cacchiarelli: Conceptualization, Methodology, Investigation, Writing – original draft, Writing – review & editing. **Filippo Fratini:** Conceptualization, Methodology, Investigation, Writing – review & editing. **Monica Puccini:** Writing – review & editing. **Sandra Vitolo:** Writing – review & editing. **Gisella Paci:** Writing – review & editing. **Simone Mancini:** Conceptualization, Methodology, Formal analysis, Writing – original draft, Writing – review & editing, Funding acquisition.

Declaration of competing interest

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